Occurrence of Antibiotic-Resistant and Plasmid DNA Harbouring Bacterial Pathogens in Stressed Polluted Water Environment of Lake Manzala, Egypt

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Abstract: This study aims at characterization of microbial pollution of Lake Manzala, bacteriological investigation of water and fish and isolation of antibiotic resistant and plasmid harbouring bacterial strains. The study revealed high levels of pollution in the water and fish samples taken from the most important sites (Kapoty, Bashtier and Matarya areas), representative of human activity and the different ecosystems in the Lake water environment. The testing for total suspended solids (TSS), ammonia and nitrates, demonstrated that figures exceeded national and international standards. The fish-tissue samples gathered from two different sites yielded high concentration of bacterial count by plate count method. Total viable bacteria (TVB) reached more than $10^6$ cfu mL$^{-1}$ in water samples and $10^5$ cfu g$^{-1}$ in fish samples, particularly in Kapoty and Matarya areas. Faecal coliform counts reached $10^6$ cfu mL$^{-1}$ in water samples and $10^5$ cfu g$^{-1}$ in fish samples. The API-20E test kit was used for the identification of eighty isolates of different bacteria strains. The bacterial strains Stenotrophomonas maltophilia, Proteus mirabilis, Escherichia coli and Erwinia sp. were common species found in the samples of the study and demonstrated multi-drug resistance. These strains harbored β-Lactamases and plasmid DNA; characteristics that can be attributed to the stressed water environment of the polluted Lake Manzala.

Key words: Water pollution, Lake Manzala, Stenotrophomonas maltophilia, Proteus mirabilis, antibiotics resistance, plasmid DNA

INTRODUCTION

Lake Manzala is a shallow coastal lake that consisting of thirty basins with depths ranging from 0.7 to 3 m in depth, the deepest areas rest in the navigation canal (Zaky, 2006). It is situated east of the Nile River's Delta, between the Damitta branch of the Nile River and the Suez Canal. The Mediterranean Sea is immediately north of the narrow peninsula which separates the two bodies of water. Lake Manzala did not exist before the 6th century AD, before then it was an area of cultivated land with fertile soil, tectonic plate movement caused an earthquake that created the lake (Zaky et al., 2005). Creation of canals and drains such as the Bahr El-Bakr drain, the Sirw drain, the Ramsis drain and the Hadous drain has created a eutrophic condition and low salinity levels in the lake. The areas around the drain outlets in the south and west are characterized by brackish water and the areas in the northeast, near the sea outlets, are saline (Zaky, 2006).

The lake suffers from water pollution induced by agricultural drainage, industrial wastes and sewage and has been shown to be contaminated by persistent organochlorine pollutants (Abbassy et al., 2003). This pollution condition of the lake has increased bacterial content particularly that of pathogenic bacterial indicators, such as the fecal coliforms, E. coli, Enterococci and Clostridium perfringens and is manifested in the water as well as in the fish populations (El-Sarangawy, 1990;
Zaky, 2006). Pathogenic species such as, *Aeromonas hydrophila* and *Aeromonas sobria*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Vibrio anguillarum*, were present in the gills, intestines and flesh of the fish samples. The specimens exhibited toxigenic characteristics as well as multi-drug resistance which could explain the marked reduction of fish and the increase of diarrheal diseases among human populations residing in the north eastern coast of Egypt (El-Gaber et al., 1997; Sulayman, 1999; Zaky et al., 2005). This study aims at characterization of microbial pollution of Lake Manzala, focusing on the antibiotic resistant bacterial strains.

**MATERIALS AND METHODS**

From April to July of 2003, 10 water samples were taken from Kapoty, Bashtier and Mataryia areas, fish samples were collected from El-Bashhtier Area and El-Mataryia Area. The selected sites are host to significant populations around the lake and receive various types of pollutants which negatively affect the condition of the lake and human health.

**Sampling Sites**

El-Kapoty area samples were taken from the end of the junction canal, which connects the Suez Canal with Lake Manzala, the main source of pollution in this area comes from the city of Port-Said. Effluents such as sewage water and industrial wastes from multiple factories are disposed of in this area of the Lake. This site is close to El-Kapoty village, a fishing village in Port-Said, where they dispose of raw sewage directly into the Lake water. El-Mataryia area is considered the fresh-water part of the Manzala Lake; however, it receives high amounts of different types of pollutants. Untreated sewage from the El Dakahlia governorate empties here as well as 6 million m3/day of industrial and agricultural waste from the El-Siwa, Hadous, Ramsis and Bahr El-Bakar drains. The drains empty into the El Genka reservoir, a part of the lake that is characterized by vast vegetation composed of reeds and other aquatic plants like the water hyacinth, this area is particularly important for fishing, specially the fishing of *Tilapia* sp. El-Bashhtier area is considered a mid point between the El-Kapoty and the Mataryia areas; it receives water currents from different directions resulting in high water levels. The depth reaches three meters and is part of the navigation canal. The area has many islets which are inhabited by people who work in fishing and raise animals (Fig. 1).

** Sampling Methods**

Ten water samples from each site were taken in clean sterile one liter glass bottles and transported from the lake to the laboratory within 6 h. The bottles were kept in ice bags and ice jackets for direct examination. Ten fish samples were taken from the El Bashhtier area and the El Mataryia area. Once the fish samples were collected they were immediately packed in sterile polyethylene cases and preserved in ice. All samples collected were transported, homogenized and prepared for immediate bacteriological analysis, using 0.8% saline for the pour plate method.

Water samples were directly diluted with 0.8% saline distilled water for bacterial counting using the dilution method and pour plates method. The fish were dissected and 1 g of the intestinal contents was aseptically stripped out with sterile forceps. Samples of gill lamellae and intestinal contents (1 g) were aseptically re-suspended in 100 mL of 0.1% w/v chilled peptone water (pH 7.0), homogenized and poured with different dilutions into Petri dishes (Zaky et al., 2005).

**Physicochemical Characteristics of Water Samples**

Physicochemical characteristic of each water sample were evaluated in the field. The temperature of the water samples was determined by using a mercury thermometer. The pH values were determined using a recombination of a pH electrode (Ell Series 1180) and a pH meter (Ell, Model 7030), which
was calibrated against pH 4, 7 and 9 buffers. The salinity of the water was determined using a refractometer model Leica No. 10419. The levels of dissolved oxygen in the water samples were determined using the Oxygen Meter model YSI 58. The chemical analysis to determine the total suspended solids (TSS), ammonia, nitrates, calcium, magnesium and chloride levels were done using the recommended standard methods of water analysis (APHA, 1995).

**Microbiological Analysis**

Counts of total viable bacteria were determined utilizing nutrient agar with 5 g of peptone, 5 g of sodium chloride, 3 g of beef extract and 15 g of agar L\(^{-1}\) of distilled water. The incubation period was 24 h and the temperature was kept at 37°C. Counts of faecal coliforms were estimated utilizing endo agar medium was composed of 10 g of lactose, 10 g of peptone and 3.5 g of dipotassium hydrogen phosphate, 2.5 g of sodium sulfate, 0.5 g of basic fuchsin and 15 g of agar L\(^{-1}\) of distilled water. The incubation period was 24 h and the temperature was kept at 44°C. Bacterial isolates were further identified by taking typical colonies from the agar medium and identifying them according to the recommended API 20 E system (bioMerieux) for identification of the family Enterobacteriaceae.

**Resistance to Antimicrobial Agents**

The disc diffusion method was used to find antimicrobial resistance. The bacterial strains were grown overnight in nutrient broth, then 1 mL of the broth was poured into a Petri dish and finally the
nutrient agar media (50°C) was poured. Different antibiotic discs (oxoid) were inserted into the plates, before the complete solidification of the media and the plates were incubated at 37°C for 24 h. The absence of a colony was an indication of the resistance of the bacteria to the following antibiotics: chloramphenicol (30 mcg), ampicillin (10 mcg), penicillin G (10 mcg), streptomycin (10 mcg) and Gentamycin (10 mcg) (Zaky et al., 2005).

Isolation of Plasmid DNA

The alkaline lysis method was used in mini preparation (Maniatis et al., 1982). For antibiotic resistant bacterial strains isolated from water and fish samples.

RESULTS

Physicochemical Properties of Water

Physical and chemical properties were estimated for all water samples collected from the El-Kapotty, El Bashiter and El Mataryia sites seasonally from autumn to summer. Most samples taken from these sites exceed the limits of pollution standards, such as total suspended solids TSS, ammonia and nitrate, respective to their different levels of salinity (Table 1).

Bacteriological Characteristics of Water

Bacterial counts were high in most of the water samples, particularly in those belonging to the El Mataryia area. The mean value of total viable bacterial counts reached more than 15,000 cfu mL⁻¹ and the mean value of fecal coliform counts reached more than 200 cfu mL⁻¹ (Table 1).

Seasonal variation in bacterial counts was obvious where, Summer is the highest season. Total viable bacterial counts for all sites reached a mean value of 33,733.33 cfu mL⁻¹ (SD=20,096.10). Summer fecal coliform counts (Table 2) for all sites reached a mean value of 776.33 cfu mL⁻¹ (SD=1,163.83).

Table 1: Mean values of the physicochemical parameters of the water in the different sampling sites of Lake Manzala

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Kapotty</th>
<th>Bashiter</th>
<th>Mataryia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>21.00±0.00</td>
<td>22.00±0.00</td>
<td>20.00±0.00</td>
</tr>
<tr>
<td>pH</td>
<td>8.40±0.12</td>
<td>8.10±0.12</td>
<td>8.50±0.90</td>
</tr>
<tr>
<td>Salinity</td>
<td>18.25±1.22</td>
<td>9.00±1.08</td>
<td>4.00±0.88</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L⁻¹)</td>
<td>1.975±1.00</td>
<td>1.93±0.00</td>
<td>1.97±0.00</td>
</tr>
<tr>
<td>Total suspended solids (mg L⁻¹)</td>
<td>139.50±8.19</td>
<td>81.50±8.39</td>
<td>61.00±8.25</td>
</tr>
<tr>
<td>Nitrate (mg L⁻¹)</td>
<td>0.45±0.02</td>
<td>0.50±0.20</td>
<td>0.80±0.11</td>
</tr>
<tr>
<td>Nitrite (mg L⁻¹)</td>
<td>0.15±0.02</td>
<td>0.12±0.00</td>
<td>0.30±0.18</td>
</tr>
<tr>
<td>Chlorides (mg L⁻¹)</td>
<td>4.32±0.87</td>
<td>2.05±2.10</td>
<td>1.06±1.45</td>
</tr>
<tr>
<td>Total viable bacteria (cfu mL⁻¹)</td>
<td>3.500.00±7.57</td>
<td>4.350.00±9.26</td>
<td>15,190.00±313.23</td>
</tr>
<tr>
<td>Fecal coliforms (cfu mL⁻¹)</td>
<td>54.40±5.31</td>
<td>41.29±6.69</td>
<td>212.50±1.08</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SD

Table 2: Mean values and standard deviation of different bacterial counts (cfu g⁻¹) in different anatomical parts of fishes in tow sampling sites of Lake Manzala

<table>
<thead>
<tr>
<th>Site</th>
<th>Total viable bacteria</th>
<th>Fecal coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bashiter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>1.422±10.26</td>
<td>64.25±15.50</td>
</tr>
<tr>
<td>Gill</td>
<td>607.5±7.66</td>
<td>33.75±5.23</td>
</tr>
<tr>
<td>Flesh</td>
<td>460.8±12.91</td>
<td>28.75±6.67</td>
</tr>
<tr>
<td><strong>Mataryia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>12,500.04±851.47</td>
<td>65.00±10.00</td>
</tr>
<tr>
<td>Gill</td>
<td>1,487.54±67.55</td>
<td>25.50±5.29</td>
</tr>
<tr>
<td>Flesh</td>
<td>2,830.0±1178.42</td>
<td>12.00±3.16</td>
</tr>
</tbody>
</table>

Data expressed as Mean±SD
Bacteriological Characteristics of Fishes

There were obvious variations found in the total viable bacteria and fecal coliform counts in the different anatomical parts of the fish and by site sampled. The highest count mean value recorded of total viable bacteria was 14000 cfu g⁻¹ and was recovered from the fish of the Mataryia area (Table 3).

Identification of Bacterial Strains

A total of 80 isolates were identified using the API 20E system. The following bacterial strains were found: Stenotrophomonas maltophilia 18%, Proteus mirabilis 16%, Escherichia coli 14%, Erwinia sp. 13%, Citrobacter freundii 12%, Pseudomonas sp. 10%, Sphingomonas paccini 6% (Fig. 2).

DISCUSSION

There are many studies that have looked at the polluted condition of Lake Manzala and the deterioration of the physicochemical and bacteriological conditions which have adversely affected the Lake’s ecosystem. Including the evolution of the lake water from marine brackish to brackish fresh. The eutrophication of Lake Manzala has been in response to increased freshwater inputs and
nutrients from agricultural run-off and urban waste disposal (Shaheen and Youssef, 1978). This has resulted in a dynamic ecology of Lake ecosystem, which was noted in the changing of the fish population from a mixed brackish water species to a population that is primarily constituted of a single species of Tilapia (Khalil, 1990).

The effluents of fresh water from drains like the Bahir El-Bakar drain, oppose the current from the Mediterranean Sea resulting in slow water movement to the point where the lake is almost stagnant or at least without currents (Zaky et al., 2005). The heavy loads of suspended solids and organic matter have raised the bed of the lake and have created shallow water depths. The high densities of microbial populations are noteworthy, particularly those of bacteria.

This study revealed high records of pollutants, like ammonia and nitrates, the presence of these can be explained by the anoxic conditions created by the depletion of oxygen. In addition high suspended particles whether of organic or mineral origin, adsorb bacteria to their surfaces. The adsorption of debris particles not only provide microbes a more favorable nutritional environment than those found in free water but also neutralize inhibitory and toxic substances. Thus the suspended particles have a favorable effect on the bacterial growth.

Fecal coliform counts, well known water pollution indicators, were performed on the different anatomical parts of the fish samples from the Kapoty and Matarya areas. These counts were used in the study to assess and understand the level of pollution of Lake Manzala.

The classical method for the enumeration of heterotrophic bacteria was used in the study; this method accomplishes the separation of individual microbes from the multitude of micro-organisms in a natural microbial community allowing for the individual phenotypes to be determined, which is the basis of the pure culture method and are the mainstay of microbiology (Levin et al., 1992).

The deterioration of the environmental conditions in Lake Manzala can be attributed to the people inhabiting the region and their role as an element of the ecosystem. The complaints and ever increasing recorded cases of diarrhea, gastroenteritis, kidney failure and other diseases may be partially explained by the aforementioned lake condition. The Lake Manzala water samples as well as the fish samples were found to have very high pathogenic bacteria contents; some of these pathogens produce dangerous extra cellular products that are virulent. It is worthy to note that many of these pathogens belong to different taxonomic groups like the families Enterobacteriaceae and Vibrionaceae, with species such as *E. coli*, *Salmonella* sp., *Shigella* sp., *Vibrio* sp. and *Aeromonas* sp. These pathogens have been reported to exist in Lake Manzala (Uerba et al., 2003; Zaky et al., 2005).

The statistical analysis of earlier studies revealed a strong correlation between counts of *Aeromonas* sp. and fecal coliforms in Lake Manzala, a brackish water environment. Thus *Aeromonas* sp. can be a powerful tool for the assessment of the microbial pollution in the brackish water environments (Zaky, 2006; Zaky et al., 2005).

A study (Dumontet et al., 2003; Biancotti et al., 2004) revealed that *Aeromonas hydrophila* exhibited a resistance to a number of antibiotics, particularly to ampicillin and penicillin. The resistance is an indication of the presence of β-Lactamases which are common in bacterial pathogens found in polluted water environments and the Egyptian environments. These environments are rich in nutrients like calcium, magnesium and chlorides; there is a strong correlation between the existence of these nutrients and *Aeromonas* sp. counts in brackish water environments (Zaky, 2006).

This study, revealed the occurrence of different multi-drug resistant strains of *Pseudomonas* sp., *E. coli*, *Proteus mirabilis* and *Stenotrophomonas maltophilia*. The latter was isolated for the first time from polluted water in Egypt through this study; the organism is an important opportunistic pathogen that is isolated world wide from the rhizosphere of some plants, waste water and drinking tap water (Crichley et al., 2003; Hoefel et al., 2005).

*Stenotrophomonas maltophilia* is used in biotechnology for biological control of plant pathogens and bioremediation (Berg et al., 1999). Recently it has been recorded however, that its unregulated
presence in natural environments is dramatically increasing, causing bacteremia, pneumonia, infections of the urinary tract and soft tissues, as well as many nosocomial infections in children and adults (Boktour et al., 2006; Deletro et al., 2006). The presence of this organism poses a risk to the health of the populations residing along the lake in this important coastal area of Egypt. The organism harbours a DNA plasmid and is β-Lactamase resistant characteristics which are of primary importance in the epidemiology of infectious diseases and clinical (nosocomial) infections. More studies which focus on the transmission of opportunistic bacterial pathogens like Stenotrophomonas maltophilia are needed.

REFERENCES


